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Brief Research Communication

Additional Clinical and Cytogenetic Findings Associated With Rett Syndrome

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An analysis of all aphidicolin-inducible breakpoints has been carried out in PHA stimulated T-lymphocytes of five patients with classical Rett syndrome, their mothers and a group of age matched controls. Observed breakpoints were divided into two groups: common, rare, and those recorded by others but not assigned as fragile sites by CCM92 and a group of non-specified breakpoints recurrently found in our ongoing study of fragile sites. In addition co-occurrence of trisomy X in one patient and de novo pericentromeric inversion on chromosome 2 in another Rett syndrome patient are reported. The co-occurrence with the Tourette syndrome in two of our families, and the fact that both Rett and Tourette syndrome are associated with movement disorders, possible dopaminergic hypersensitivity and increased chromosomal fragility in subsets of fragile sites, may suggest a possible avenue for further research. The cytogenetic findings indicate that both X-linked and autosomal regulatory region(s) may be part of a complex genetic alteration in association with Rett syndrome. Am. J. Med. Genet. 74:331–337, 1997.

KEY WORDS: Rett syndrome; fragile sites; aphidicolin; chromosome aberrations; Tourette syndrome

INTRODUCTION

Rett syndrome (RS) is a neurologic disorder noted in females, characterized by a loss of communication skills, purposeful hand use, and the appearance of stereotypic hand wringing movements. Recently an expanded phenotype has been described [Percy, 1995].

The few familial cases observed suggest a genetic susceptibility to the syndrome. Since Rett syndrome has been observed in females only, genetic studies have focussed mainly on the X-chromosome and the hypothesis mostly favoured is X-linked dominant inheritance which is lethal in males. There may well be an even more complicated mode of inheritance, with not only involvement of the X chromosome but also an autosomal locus [Anvret et al., 1993, 1994].

Increased chromosome breakage in RS patients at many chromosomal sites after incubation of T-lymphocytes in folic acid deficient medium has been reported by Telvi et al. [1994]. In addition contradicting reports have been published about the relationship between RS and increased expression of the common fragile site at Xp22.3 [Wahlstrom and Anvert, 1986; Wahlstrom et al., 1990; Gillberg et al., 1984, 1985; Romeo et al., 1986].

Fragile sites (FS) on human chromosomes can be defined as vulnerable regions where different kinds of lesions preferentially occur either spontaneously or after induction with certain breakage-inducing agents. A large number of common FS are induced after exposure to aphidicolin, an inhibitor of the DNA polymerase alpha [Glover et al., 1984]. Several lines of information appear to converge towards a conclusion that FS may be a manifestation of chromatin alterations taking place in or near genetically active areas [Simonic and Gericke, 1996]. If so, then differences in expression of certain breakpoints on chromosomes in RS patients may represent an altered state of genetic activity within the area when compared to age matched controls, family members, and the general population. On the other hand, a general increase of expression in most of the breakpoints may suggest the existence of a genetic factor that favours chromosome breakage in RS. For this reason we investigated aphidicolin-induced fragile site expression in available patients with Rett syndrome together with siblings and parents where available, as well as matched controls, to ob-

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TABLE I. Frequency of Common Aphidicolin-Inducible Fragile Sites for Rett Syndrome Patients (RS), Their Age Matched Controls (Control-1), Their Mothers (Ma's) and Female Controls in the Same Age Group (Control-2)

Group 1 site	RS mean	RS SD	Control 1 mean	Control 1 SD	Ma's mean	Ma's SD	Control 2 mean	Control 2 SD
1p36	3.40	1.62	2.60	1.20	3.25	0.83	2.75	2.28
1p32	8.20	4.07	4.40	2.15	5.00	1.22	4.50	0.87
1p31	10.00	5.14	10.40	3.93	3.50	1.80	4.00	0.71
1p22	1.60	0.80	1.20	1.17	0.75	0.43	0.50	0.50
1p21	20.80	6.97	14.20	3.19	18.75	1.48	12.00	4.30
1q21	0.60	1.20	0.60	0.80	1.25	0.83	0.25	0.43
1q25	7.20	3.71	3.80	0.40	5.25	3.11	4.00	2.55
1q31	2.60	1.20	0.40	0.49	0.00	0.00	0.50	0.87
1q42	1.40	0.80	1.60	1.50	0.75	1.30	0.50	0.50
1q44	29.60	6.34	21.60	5.85	20.00	5.87	17.25	6.30
2p24	15.80	6.94	13.40	4.50	16.25	5.76	12.25	8.87
2p16	4.60	1.74	3.80	1.83	6.50	2.87	3.50	4.92
2p13	5.20	4.71	4.60	2.58	1.75	0.43	3.50	0.50
2q13**	0.60	0.80	0.40	0.80	0.50	0.50	0.50	0.50
2q21	8.00	3.63	4.60	1.96	8.25	1.30	6.00	4.06
2q31	3.20	2.14	2.20	2.14	1.67	1.70	2.25	1.48
2q32	12.60	3.44	7.40	4.03	14.50	5.68	9.00	1.87
2q33	6.20	2.14	4.40	2.58	4.00	1.58	5.25	1.30
2q37	9.40	3.38	6.00	2.45	12.50	3.20	10.00	3.67
3p36*	11.00	3.29	5.80	1.60	7.25	2.49	6.75	1.48
3p24	5.80	2.32	5.00	2.28	5.00	1.00	3.50	1.12
3p21*	4.00	1.41	1.80	1.17	3.00	0.71	1.00	1.00
3p14	81.00	13.18	68.60	7.31	62.00	6.04	58.25	11.03
3q13*	7.20	3.54	5.80	1.47	6.25	2.28	3.00	2.12
3q21*	0.80	0.75	1.20	1.17	1.25	1.30	1.00	0.71
3q25	2.00	0.89	0.60	0.80	3.00	1.58	3.00	1.58
3q27	6.00	1.41	6.40	3.61	4.25	2.77	2.00	1.22
4p16	5.60	1.50	4.20	2.23	4.50	0.50	2.75	1.30
4p15	2.00	2.28	2.20	1.47	3.00	1.22	1.75	2.05
4q21	1.60	1.62	1.40	1.36	2.00	1.87	1.00	0.71
4q23*	4.00	2.68	1.80	1.17	3.00	0.00	3.75	0.83
4q27*	1.40	0.80	0.40	0.49	1.50	1.12	1.75	0.83
4q31	14.60	4.59	10.20	2.32	13.50	2.06	12.75	2.86
5p14	1.60	1.36	4.60	4.13	4.25	1.64	2.00	2.35
5p13	3.60	2.06	3.20	2.04	2.50	1.12	3.00	1.73
5q15	5.00	2.53	4.80	2.56	5.00	3.16	3.00	1.87
5q21	1.60	1.20	1.00	0.89	2.50	0.87	1.25	1.30
5q31	0.20	0.40	1.00	1.55	0.75	0.83	0.75	0.83
6p25	16.00	4.29	11.40	1.74	12.75	0.83	15.00	8.06
6p22	1.60	1.02	1.80	1.72	1.25	1.09	1.00	0.71
6q15	1.60	1.62	1.60	1.02	3.00	1.58	1.50	2.06
6q21	7.00	2.76	2.40	1.62	3.00	1.22	5.25	4.55
6q26	20.20	9.02	12.60	4.84	8.50	3.20	8.25	3.42
7p22	14.40	6.34	13.60	6.89	14.00	5.43	13.00	7.35
7p14	2.40	1.62	1.00	1.26	2.00	1.41	1.25	1.09
7p13	17.00	4.69	7.80	5.00	14.75	3.56	12.00	4.53
7q11	6.80	1.94	6.20	3.43	6.75	1.92	6.75	1.92
7q21	6.40	2.58	2.40	1.02	3.25	2.38	4.50	2.69

serve what further information could be obtained in this manner.

MATERIAL AND METHODS

Whole blood samples have been collected in heparinized tubes from five caucasian girls with the classical form of Rett syndrome diagnosed by two pediatricians according to established diagnostic criteria [Percy, 1995]. In four instances, samples from parents and available siblings and additional specimens from four age and sex matched controls to the mothers of RS girls and five age and sex matched controls to the probands have been included for comparison. Only specimens from females were used for the final evaluation. The male members of the families served as controls for the possibility of inherited changes in FS expression. In the family of the patient with *inv2,* the father and one of the two brothers were diagnosed as having Gilles de la Tourette syndrome (TS) by means of the DSM IV criteria.

In another instance a proband's cousin from the father's side was diagnosed positive for RS but died at an early age. In this family the mother, father and brother of the proband fulfill the criteria for TS.

One girl has been identified in an institution for mentally disabled and her blood was sent for routine cytogenetic analysis to our laboratory. According to the records her parents are consanguineous and were not available for the study. All individuals included in the study except the *inv2* and trisomy X probands had normal karyotypes.

TABLE I. Continued

Group 1 site	RS mean	RS SD	Control 1 mean	Control 1 SD	Ma's mean	Ma's SD	Control 2 mean	Control 2 SD
7q22	1.60	0.80	1.20	1.17	0.75	0.83	1.50	1.12
7q31	15.40	5.50	8.80	2.14	9.00	1.73	7.50	3.28
7q32	22.80	11.60	13.80	2.79	17.50	2.69	18.25	7.82
7q36	0.80	0.75	1.00	0.63	0.25	0.43	1.00	1.00
8q21**	1.00	0.63	0.80	0.75	0.25	0.43	0.25	0.43
8q22	11.40	3.07	5.20	2.04	12.00	3.32	6.25	6.26
8q24.1	3.40	1.36	3.40	1.74	6.25	1.79	3.50	0.50
8q24.3	5.80	1.17	3.00	1.26	5.75	3.27	3.25	1.09
9p21	3.40	2.58	1.60	2.73	2.00	1.58	0.75	0.43
9q12	0.60	1.20	0.60	0.49	0.50	0.50	0.50	0.87
9q22	2.20	1.47	1.20	1.47	0.75	0.83	1.25	0.83
9q32	14.40	1.62	11.40	2.42	16.25	3.27	12.25	4.44
10q21	1.80	0.75	0.00	0.00	0.75	0.43	0.50	0.87
10q22	2.40	0.80	2.00	2.10	2.50	1.50	1.75	0.43
10q23**	0.60	0.80	0.40	0.49	0.50	0.50	0.50	0.87
10q25	4.20	1.72	3.80	2.40	3.25	1.79	2.75	1.30
10q26	10.80	2.93	10.20	4.79	8.00	3.67	3.50	0.50
11p15	4.00	2.19	3.40	0.80	3.00	0.71	4.00	2.12
11p14	3.20	1.72	2.60	1.62	3.00	1.00	2.25	0.83
11p13	12.20	3.31	11.60	3.83	17.75	0.43	8.25	2.17
11q13**	1.40	1.02	0.40	0.49	0.75	0.43	0.50	0.50
11q14	7.60	4.08	6.00	1.10	9.50	2.29	5.75	2.86
11q23	1.40	1.20	0.60	0.80	1.75	1.48	0.75	0.83
12q13**	2.40	1.50	1.20	0.98	0.25	0.43	0.50	0.50
12q21	4.20	3.12	4.20	1.33	5.75	1.09	1.50	1.50
12q24	2.00	2.28	1.60	0.80	1.75	1.48	1.25	1.64
13q13	13.00	4.77	6.60	2.50	5.75	1.30	5.00	3.54
13q21	1.60	1.62	1.20	1.17	1.00	1.00	0.50	0.87
13q32	2.20	0.75	1.20	1.17	1.50	1.12	1.75	1.30
13q34*	1.80	0.75	1.00	0.63	1.50	1.12	1.25	0.83
14q23	3.80	1.72	2.60	1.50	2.50	1.66	2.50	2.29
14q24	15.40	3.56	11.00	4.98	11.50	3.64	10.50	4.27
15q22	0.80	0.75	1.00	0.63	0.25	0.43	0.25	0.43
16p13**	0.60	0.80	0.80	0.98	0.75	0.83	0.50	0.87
16q22	1.80	1.60	4.60	2.33	1.50	1.50	1.00	0.71
16q23	67.00	9.38	50.40	8.69	51.50	8.62	43.50	9.01
17p12**	0.40	0.49	0.80	0.75	0.50	0.50	0.25	0.43
17q23	1.40	1.20	1.00	0.63	1.25	1.09	1.00	0.71
18q12	10.40	5.82	8.60	2.24	8.50	2.18	3.75	1.30
18q21	1.20	1.47	0.80	0.75	1.00	0.71	0.25	0.43
19q13.1	3.60	2.15	2.40	1.20	3.00	2.24	2.00	1.00
20p12	6.60	4.22	4.80	1.60	6.25	4.44	2.50	0.87
22q12	9.80	2.32	6.00	1.79	8.75	1.48	8.25	1.48
22q13**	0.60	1.20	0.40	0.49	0.25	0.43	1.75	1.30
Xp22.3	54.00	4.98	37.00	9.25	44.00	8.31	39.50	11.54
Xq22	24.40	10.33	15.00	2.76	20.50	1.50	13.25	3.34
Xq27	1.00	0.89	0.20	0.40	1.75	0.43	0.50	0.50
Xq28	1.20	1.94	0.00	0.00	0.75	0.83	0.25	0.43

*—Breakpoints detected in our study and reported by others, but not listed in CCM92 as fragile site.
**—Breakpoints considered as rare folate-sensitive fragile sites.
Mean, mean frequencies of expressed breakpoints in 100 metaphases.
SD, standard deviation.

Two PHA-stimulated lymphocyte cultures were established for each individual by using 0.5 ml of whole blood in RPMI-1640 medium supplemented with 10% FBS. After 48 hours, aphidicolin dissolved in 70% ethanol was added (24 hour treatment) to each 10 ml culture to obtain final concentration of 0.1 μM. Standard cytogenetic techniques were used for harvests and slide preparation.

Fifty complete metaphase spreads for each culture (100 per individual) at the 400–600 band level were assessed. All aberrations (gaps, breaks, and chromatid exchanges) were scored as single events at the band(s) involved. In total, 147 breakpoints were recorded and divided into two groups according to their status as common FS, rare FS [Chromosome Coordinating Meeting, 1992], breakpoints recorded by others, but not listed as common FS (Table I), and so called non-specified breakpoints repeatedly found in our analyses (Table II).

RESULTS

The computed means and standard deviations per 100 cells of all breakpoints in T-lymphocytes for investigated groups are summarised in Table I and II.

TABLE II. Frequency of Non-Specified Aphidicolin-Inducible Fragile Sites for Rett Syndrome Patients (RS), Their Age Matched Controls (Control-1), Their Mothers (Ma's) and Female Controls in the Same Age Group (Control-2)

Group 2 site	RS mean	RS SD	Control 1 mean	Control 1 SD	Ma's mean	Ma's SD	Control 2 mean	Control 2 SD
2p25	0.80	0.75	2.60	1.85	2.25	0.83	2.75	1.92
2q12	0.20	0.40	0.00	0.00	0.50	0.87	0.50	0.50
3p13	1.60	1.02	3.20	2.71	2.75	1.30	1.75	1.79
3q28	2.40	0.80	0.40	0.49	1.50	0.50	0.25	0.43
4q12	0.60	0.49	1.20	1.17	1.50	1.50	0.75	0.83
4q34–35	0.60	0.49	1.20	1.47	1.50	1.66	0.75	0.83
5q13	1.40	1.02	1.60	1.96	1.50	2.06	2.25	1.09
5q33–34	1.20	1.17	1.60	1.36	0.50	0.50	1.00	0.71
6p21.1	4.20	4.40	3.60	3.01	5.25	1.64	1.25	2.17
6q23	1.40	0.80	2.00	1.79	1.25	0.83	0.75	0.43
6q25	1.20	0.75	0.80	0.75	0.75	0.43	0.75	0.43
8p25	1.60	2.06	2.00	1.67	1.25	0.43	0.25	0.43
8p21	1.00	1.10	0.80	0.75	1.00	1.22	0.75	0.83
8q11.2	3.00	1.90	3.60	2.80	1.50	0.50	2.75	1.92
9p24	2.00	1.10	1.60	1.20	1.25	1.30	1.00	1.22
9q21	0.80	1.60	2.40	1.96	2.00	1.00	0.75	0.83
9q34	2.00	1.26	1.20	1.17	0.50	0.87	1.50	1.12
10p15	0.80	0.98	0.00	0.00	0.75	0.83	0.00	0.00
10p13	6.60	4.88	14.80	11.27	6.25	2.86	3.50	1.66
10p11.2	6.20	2.79	5.20	4.26	4.25	1.92	2.25	1.64
12p12	1.60	1.02	1.20	1.17	0.50	0.50	0.75	0.83
12q24.3	0.40	0.80	0.80	0.98	0.75	0.43	0.25	0.43
14q12	1.00	0.89	0.40	0.49	0.50	0.87	0.25	0.43
14q13	6.00	3.46	4.00	3.03	3.25	0.43	2.00	1.87
14q31	0.80	0.75	0.00	0.00	0.50	0.50	0.75	0.83
14q32	0.40	0.49	0.00	0.00	0.25	0.43	0.25	0.43
15q13	0.80	0.98	2.40	1.85	2.50	1.12	1.75	1.79
15q14–15	2.00	2.10	1.60	1.50	1.50	0.50	1.75	1.92
16q21	0.80	0.40	2.40	1.85	1.75	1.09	1.00	0.71
17q21	1.60	1.02	1.20	1.17	0.50	0.50	0.50	0.50
17q25	2.60	1.36	3.60	2.80	2.25	1.30	1.50	0.87
18p11.2	8.60	5.57	10.00	7.97	1.50	1.50	1.25	1.30
18q11.2	0.20	0.40	0.00	0.00	0.25	0.43	0.00	0.00
18q22	0.80	0.75	0.80	0.75	0.50	0.50	1.25	1.30
18q23	1.40	1.36	0.00	0.00	1.25	0.83	0.25	0.43
19q13.3	0.20	0.40	0.40	0.49	0.25	0.43	0.00	0.00
20q11.2	1.80	0.40	0.80	0.75	1.00	0.71	0.50	0.50
20q13.3	0.60	0.80	0.00	0.00	0.50	0.50	0.00	0.00
21q22	1.00	1.26	2.00	1.55	1.75	1.92	1.00	0.71
22q11.2	0.20	0.40	0.00	0.00	0.50	0.50	0.00	0.00
Xp22.1	7.40	3.72	3.20	3.25	1.75	1.30	1.25	0.83
Xp11.4	0.60	0.49	0.00	0.00	0.00	0.00	0.00	0.00
Xp11.2	1.20	1.17	0.80	0.75	0.50	0.50	0.25	0.43
Xq12	1.00	0.63	1.60	1.36	0.75	0.83	1.25	0.83
Xq13	3.80	3.31	5.60	4.59	4.50	2.29	1.00	0.71
Xq24	0.60	0.80	0.40	0.49	0.00	0.00	0.00	0.00
Xq26	1.00	0.89	0.00	0.00	0.50	0.87	0.25	0.43

mean—mean frequencies of expressed breakpoints in 100 metaphases.
SD—standard deviation.

No statistically significant increase in overall chromosomal fragility was observed by applying both parametric (t-test) and non-parametric methods [Kolmogorov-Smirnov, Wilcoxon sign, and ranks and pair (Mann-Whitney) tests] for the net comparison between control and the two tested groups of interest (RS and Ma's). The value of this observation is unclear because of a very small available sample size and relatively high standard deviations for individual fragile sites. The increase of fragility in favour of the RS group vs Control-1 and Ma's vs. Control-2 was only indicated when using Wilcoxon sign and ranks tests ($P = 0.05$). Although the statistical value of these methods is lower than the ones which gave negative results, the positive findings may suggest that increasing the sample size may result in different outcomes.

The frequency of individual breakpoints was not evaluated statistically. Nevertheless there were breakpoints observed with obvious differences of expression in RS group opposed to the others.

The most intriguing finding is the expression of *Xp22.1* (Fig. 1) breakpoint in RS patients in 13%, 9%, 8%, 5%, and 2% of the metaphases analysed. The lowest count was found in the girl with *inv2.* In patient with trisomy X, the breakpoint was counted in 8% of the metaphases. The only case when the abovementioned breakpoint was observed in considerably higher frequency (6%) within controls was the sample

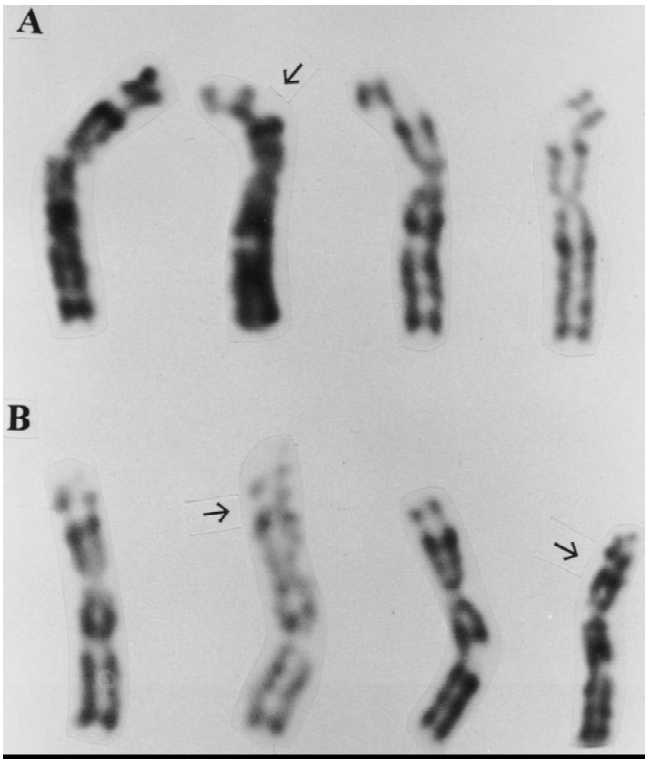

Fig. 1. **A**: *Xp22.1* breakpoint. **B**: *Xp22.1* gap.

of the 3-year-old healthy girl with a TS father and mother with familial B12 deficiency. In the specimens from two TS brothers of probands the breakpoint was observed in 4% and 3% of the metaphases.

The common fragile sites at *3p14, 16q23,* and *Xp22.3* were observed more often in RS patients than in other groups confirming the published study of Telvi et al. [1994]. Considerably increased breakage rates in RS versus age matched controls have been found in other areas as well (Tables I and II).

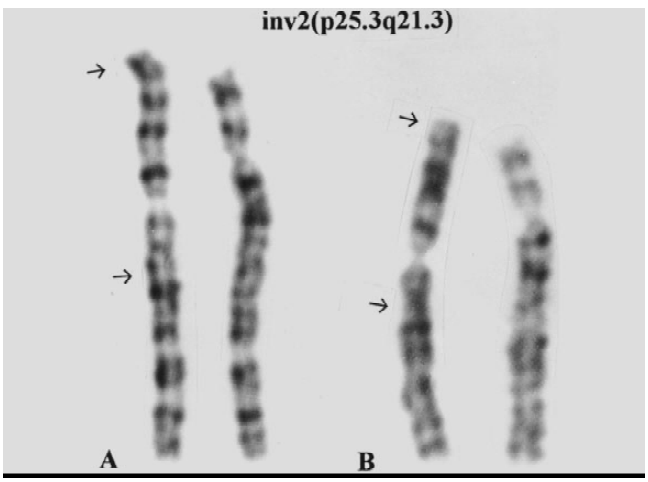

Fig. 2. **A**: *Inv 2 (p25.3 q21.1)* at the 750 band level. **B**: *Inv 2 (p25.3 q21.1)* at the 600 band level.

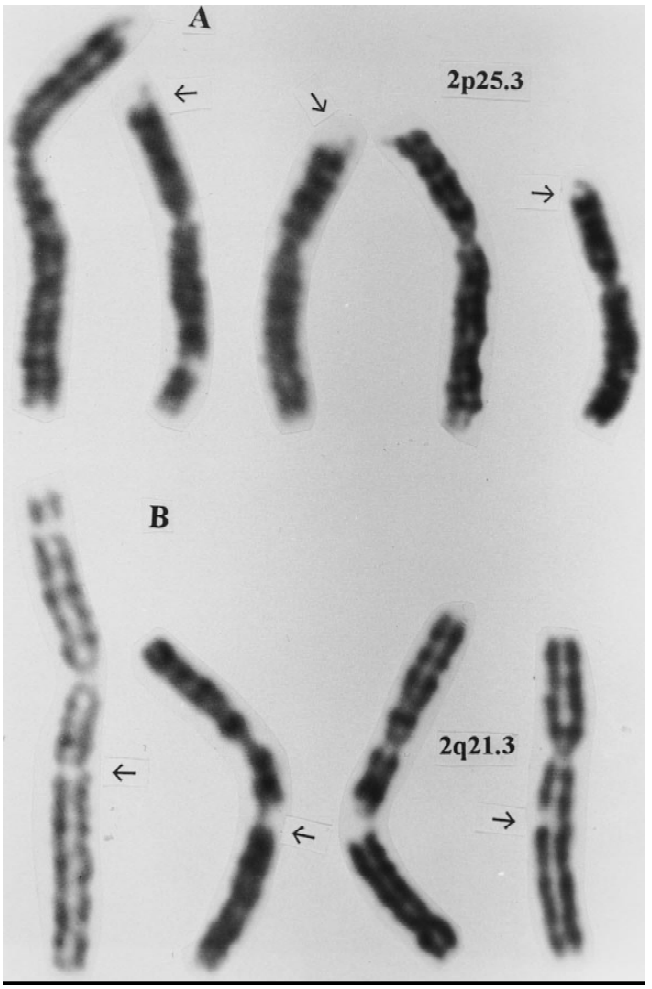

Fig. 3. **A**: *2p25.3* breakpoint. **B**: *2q21.3* breakpoint.

The de novo pericentromeric inversion on chromosome 2 was identified as *inv2(p25.3q21.3)* (Fig. 2). There is a strong possibility that breaks and reunions in this structural abnormality emerged within the fragile sites at *2p25* and *2q21* (Fig. 3).

DISCUSSION

While fragile sites are highly conserved throughout evolution and the rank orders of their expression under specified culture conditions are similar in each individual, other mechanisms than simple inhibition or late replication must also be involved in their expression. Several authors have suggested that fragile sites might represent amplification of naturally occurring polypurine/polypyrimidine sequences [Sutherland and Hecht, 1985; Nussbaum et al., 1986]. These types of microsatellite sequences have been found in non-exon areas of the genes and their role in the human genome remains enigmatic. The resistance of autosomal heterochromatin of *Peromyscus* to aphidicolin-induced chromosomal breakage [Dominiguez et al., 1995] argue against a simple relationship between late replication and a general mechanism for chromosomal fragility.

Our study was too limited to evaluate individual fragile site expression statistically, but clearly not all the sites evaluated were expressed with higher frequencies in RS when compared to age matched controls. That opens the possibility of factor(s) being involved which alter the susceptibility to aphidicolin-ethanol treatment in only a subset of common fragile site areas. Similar findings have been documented in Tourette syndrome [Gericke et al., 1996; Simonic and Gericke, 1995], although in a different subset of common fragile sites.

The most intriguing observation in our study is the high frequency of the *Xp22.1* breakpoint in the RS samples. Its precise localization on molecular level would be of interest, while co-occurrence of a new *t(X;3)(p22.11;q13.31)* with Rett syndrome was described [Zoghobi et al., 1990] and the breakpoint on X chromosome revised to *Xp21.3* by YAC cloning [Ellison et al., 1992; Curtis et al., 1993]. If there is a gene responsible for Rett syndrome etiology localised in *Xp21.3 - Xp22.1* and its mutation is represented by increased fragile site expression, then there is a high probability that the defect would be in the non-coding region of the gene.

RS is not caused by any specific chromosome aberration. Structural chromosomal aberrations such as [dup(6)(p11.2)] and [t(16;17)(p13;q21)] [Wahlstrom and Anvret 1986] segregated in families and observed in RS girls have been described. The pericentromeric inversions on chromosome 2 were reviewed by Djalali et al. [1986], but none was assigned as *inv2(p25q21).* The probability that the clinical and chromosomal abnormality co-occur just by a chance is therefore very low. It is of interest that some of the genes localized in 2p25 and 2q21 areas [McKusick, 1994] may represent good candidates for involvement in RS pathology.

Birth surveys have shown that approximately 0.1% of all females have a 47,XXX karyotype and the prevalence of RS in its classical form is 1:15,000. Taking this into account, the co-occurrence of both conditions in one of our patients may not be of particular importance, but may also suggest the RS gene to be on the X chromosome.

Despite the high prevalence of Tourette syndrome in some populations, we find the co-occurrence of these two syndromes within families of interest. The presence of a significant movement disorder in Rett syndrome, the finding of an upregulation of D2 receptors with single photon emission computed tomography in Rett syndrome [Percy, 1995], the suggestion of similar changes in the dopaminergic system in Tourette syndrome [Jankovic, 1993], and the reported increased chromosomal breakage in both conditions [Telvi et al., 1994; Gericke et al., 1996] point towards interesting areas of overlap between these two conditions which require further elucidation.

CONCLUSIONS

The unique co-occurrence of structural and numerical chromosomal abnormalities in two patients with RS, an intriguing altered expression of some fragile sites compared to controls, the variety of Rett syndrome and Rett syndrome-like (form fruste) phenotypic expressions, and the presence of other neurobehavioural and neuropsychiatric conditions in RS families (Zhang, personal communication) may suggest that a number of genes, both X-linked and autosomal, may contribute to the disease outcome. In such a case and because there are only a few families with more than one affected individual identified worldwide, it may prove useful to adopt other than classical linkage approaches in a search for the genetic basis of the syndrome.

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